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Intracellular Retention of Cytosine Arabinoside Triphosphate in Blast Cells From Children With Acute Myelogenous and Lymphoblastic Leukemia

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The importance of the cellular pharmacokinetics of cytarabine triphosphate (ara-CTP) with regard to therapeutic efficacy is well established. In vitro and in vivo monitoring of pharmacokinetic parameters of leukemic blast cells were initiated in order to contribute to the pharmacological basis of optimal ara-C treatment strategies. Peripheral or bone marrow blast cells from 66 leukemic patients [51 acute myelogenous leukemia (ALL), 15 acute lymphoblastic leukemia (AML) were separated and incubated with ara-C for 1 hour and in ara-C–free medium for another 3 hours, and the intracellular formation and retention of ara-CTP was measured. In eight children who received continuous ara-C infusion for induction treatment, the ara-CTP concentration in circulating blast cells was monitored in vivo. The in vitro values observed in this assay corresponded to the cellular levels monitored in vivo. The ara-CTP retention differed clearly among the individual groups, as classified by immunphenotype at the time of the initial diagnosis: non–T-ALL 67 ± 25% (x ± SD, n = 33), T-ALL 37 ± 15% (n = 8), and AML 34 ± 18% (n = 14). The difference in ara-CTP retention between non–T-ALL and AML ($P < 0.05$) as well as T-ALL ($P < 0.05$) was significant. There was a tendency toward lower ara-CTP retention in relapsed as compared with newly diagnosed ALL, but the difference was not significant. The maximal accumulation of ara-CTP (after 1 hour incubation) was comparable in AML, T-ALL, non–T-ALL, and blast cells from children in relapse. The observed similarity of cellular accumulation in all groups and the significantly more rapid decrease in T-ALL and AML provide the pharmacokinetic rationale supporting the prolonged infusion duration for ara-C in these subgroups as an alternative to the intensification by high-dose ara-C schedules with short-term infusion.

Key words: leukemia, cytarabine, cytarabine-triphosphate, cellular pharmacology, T-ALL

INTRODUCTION

Cytarabine (ara-C) is one of the most active cytotoxic drugs in the treatment of childhood acute leukemia. Dosing and scheduling vary widely and are still subject to intense discussion. During the last decade the treatment intensity was successfully increased by high-dose schedules (2–3 g/m^2) in relapsed and refractory leukemias, and this strategy has since been included in the consolidation therapy of acute myeloid and lymphoblastic leukemias [1–3]. The current therapy protocols of the German BFM group have incorporated a variety of ara-C schedules, namely, continuous infusion of 200 mg/m^2/48 h conventional short-term infusion of 100 mg/m^2/30 min every 12 hours, low-dose IV push of 75 mg/m^2/day, and high-dose administration of 2,000–3,000 mg/m^2 over 3 hours, every 12 hours.

The pharmacological basis for the different treatment modalities has been exhaustively investigated [4–8], but the optimal mode of application has remained controversial. Cellular uptake and intracellular phosphorylation to the nucleotide cytosine arabinoside triphosphate (ara-CTP) are preconditions and determinants of the cytostatic effect of cytarabine [9]. At conventional dosages (around 100 mg/m^2), the cellular uptake of ara-C occurs by facilitated diffusion and depends on the number of transmembraneous nucleoside carrier sites [10]. At higher plasma levels that can be obtained during the 2–3 hours of high-dose ara-C therapy (2–3 g/m^2), the cellular uptake by passive diffusion occurs independent of transport capacity [11].

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Several studies reported a correlation between the leukemic cells' ability to build and to retain the metabolite ara-CTP intracellularly and the clinical response to therapy in AML [12,13]. Various strategies were proposed to custom tailor ara-C infusion regimens on the basis of intracellular ara-CTP pharmacokinetics [12,14]. Such in vivo strategies, however, are restricted to therapeutic situations with evaluable peripheral blast cell counts.

An in vitro assay introduced by Rustum and coworkers [13] showed a correlation between the ara-CTP retention of the leukemic cell and remission duration in patients with myeloid leukemia. Observations of cellular ara-CTP pharmacokinetics in lymphoblastic leukemias, especially in pediatric patients, are rare [15,16]. Hence, the present study focused on in vitro intracellular ara-CTP formation and retention. The objectives were relevant differences in cellular ara-CTP pharmacokinetics in blast cells from children with acute T- lymphoblastic, acute non–T-lymphoblastic, acute myeloid leukemia, and relapsed leukemias, which may provide a convincing background for the different dose schedules currently used in pediatric treatment protocols.

MATERIALS AND METHODS

Cell Culture

The myeloid cell lines K562 and HL60 and the pre–B-lymphoblastic cell line Blin1 [17] were used to establish and validate the incubation assay and the analytical procedures.

Separation of Blast Cells

Prior to any chemotherapy, bone marrow (2 ml) from the posterior iliac crest or peripheral blood (5 ml) was drawn into heparinized tubes as part of the routine diagnostic sampling. The samples were immediately placed on ice, diluted with 15 ml RPMI medium (containing *L-glutamine, Gibco-UK*), and the blast cells were separated by Ficoll-Hypaque (Lymphoprep™, Nycomed-Norway) centrifugation. Cells at the interface were harvested, washed three times with 50 ml medium, and centrifuged at 400 g (10 min/4°C). The cell count (Neubauer chamber) was determined, and pellets were then resuspended in RPMI- 1640 with 10% heat inactivated fetal calf serum (Boehringer-FRG) for the incubation experiments (37°C). Only samples with ≥90% blast cells were analyzed.

In Vitro Incubation Assay

The blast cell suspension was adjusted to 30–100 million cells/50 ml medium (RPMI, 10% fetal calf serum), and ara-C was added to a final concentration of 1 μg/ml. A concentration of 3 μg/ml was chosen if there were less than 30×10^7 blast cells available in order to obtain concentrations significantly above the limit of detection. In this ara- C–containing medium, the cells were incubated in a shaking water bath at 37°C for 1 hour (ara-CTP formation). The cells were then centrifuged and divided. One part was washed twice with RPMI medium and reincubated in ara-C free medium for another 3 hours (ara-CTP retention). The other part (detection of C_{1h}) and the pellet obtained after the reincubation ($C_{1 + 3h}$) were washed twice in ice-cold phosphate buffer (pH 7.2). The viability of the cells was tested to exceed 90% with trypan blue solution, and the cells were counted again immediately prior to extraction. The ara-CTP retention at the end of the second incubation period was expressed as the percent of the 1 hour level ($C_{1 + 3h}/C_{1h} \times 100$ = % retention; RT).

In order to determine the ara-CTP formation independent of facilitated diffusion, the inhibitor dipyridamole was added at a concentration of 10 μg/ml immediately before the addition of ara-C in a test series.

Reproducibility

The incubation assay was performed in triplicate on 9 subsequent days. Overall evaluation of these 27 experiments with HL60 cells showed a relative standard deviation (RSD) of 23% for C_{1h}, 28% for $C_{1 + 3h}$, and 19% for the ara-CTP retention. The day-to-day RSDs based on the evaluation of only the first experiments each day were 22%, 26%, and 18%, respectively. With fourfold incubation on a single day, the RSD of the ara-CTP retention was 14%.

Inhibition of Ara-CTP Formation by Nucleoside Transport Inhibitor

The cellular ara-C uptake by the transmembraneous carrier mechanism, and not by passive diffusion, was required in the vitro assay. Incubation of the three cell lines K562, HL60, and Blin1 for 1 hour with increasing concentrations of ara-C was associated with an increase of cellular ara-CTP. Addition of 10 μg/ml dipyridamole, a well-known inhibitor of the nucleoside carrier system [22], suppressed the ara-CTP formation up to an extracellular ara-C concentration of 10 μg/ml. In K562 addition of 10 μg/ml dipyridamole resulted in only 20% ara-CTP formation following 1 hour incubation. In HL60 and Blin1 cells, no ara-CTP was formed at all (n = 4).

Intracellular Ara-CTP

Extraction of the cells following 1 and 1 + 3 hours of incubation and quantification by high-performance liquid chromatography (HPLC) were performed as described elsewhere [18] [isocratic ion pair HPLC method with a reversed phase C_{18} column (NOVA-PAK™, Waters-FRG) and 0.09 M phosphate buffer at pH 6 containing 0.35% tetrahydrofuran and 0.01 M tetrabutyl ammonium hydrogen sulphate]. UV detection at 270 nm shows the

limit of detection at 25 ng/ml ara-CTP. Anthranilic acid is used as an internal standard in order to relate the measured concentrations to the blast cell count in the lysed cell pellet [18].

PATIENTS

From January 1989 to December 1992 the in vitro investigation of ara-CTP retention was performed with blast cells from all children and adolescents with newly diagnosed acute leukemia or relapse. Informed consent was obtained prior to bone marrow puncture, and a peripheral or bone marrow blast cell count high enough to exclude the necessity of additional sampling was required. Unsuccessful sampling of blast cells was the only exclusion criteria. Mononuclear cells from normal bone marrow aspirates were obtained from seven children tested for leukemia or to confirm their remission status.

Acute Lymphoblastic Leukemia (ALL)

Fifty-one patients aged between 3/12 and 17 years were investigated. Eight children at initial diagnosis and two in relapse were classified as T-lymphoblastic leukemia (T-ALL: TdT, CD3, and CD1 or CD7 positive). Thirty-three children at diagnosis and 11 in relapse presented with pre–pre–B-ALL (TdT and CD19 pos.), common-ALL (TdT, CD19, and CD10 pos.), or pre–B-ALL (cytoplasmatic IgM pos.), comprised of non–T-ALL. Treatment was given according to the German therapy protocols of the Berlin-Frankfurt-Münster group ALL-BFM-86 and ALL-BFM-90 [19] and to the treatment protocol for relapsed ALL [20].

Acute Myeloid Leukemia (AML)

Fifteen patients aged between 9 months and 16 years were investigated. Fourteen times the assay was performed at the time of the initial diagnosis, once both initially and at relapse, and once at relapse only. The classification according to the French-American-British (FAB) criteria showed 1 × M0, 2 × M1, 1 × M3, 4 × M2, 3 × M4, and 3 × M5. One child developed the AML as a secondary malignancy. The patients were treated according to the German AML-BFM-87 treatment protocol [21].

Intracellular Ara-CTP Monitoring

The induction therapy according to the AML-BFM-87 protocol consists of a 48-hour continuous infusion of ara-C (100 mg/m^2/day) with age-dependent intrathecal ara-C application on day 1, followed by 12 infusions of 100 mg/m^2 ara-C every 12 hours. On days 3,4, and 5 daunorubicin (30 mg/m^2) is additionally applied every 12 hours, and on days 6, 7, and 8 etoposide (150 mg/m^2) is added. Eight patients who received this induction protocol had a peripheral blast cell count high enough for intracellular monitoring of ara-CTP levels during the continuous infusion. Blood samples of 2–5 ml were then drawn into heparinized tubes, placed on ice without delay, and handled as described earlier. In two children peak and trough levels after the first ara-C short-term infusions were also monitored.

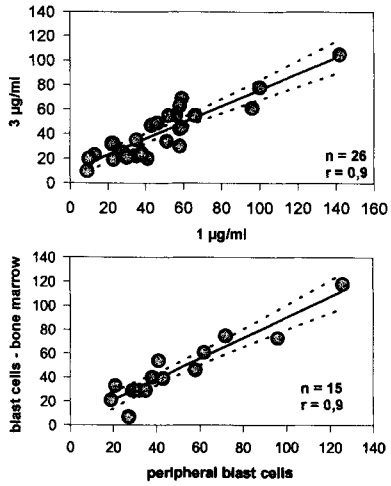

Fig. 1. Regression of the cellular ara-CTP retention in blast cells obtained from bone marrow vs. peripheral blast cells (**bottom**). Regression analysis of ara-CTP retention following incubation with 1 µg/ml vs. 3 µg/ml ara-C (**top**).

Statistics

To compare differences between the median among the immunological and morphological groups, the Kruskal-Wallis one-way analysis of variance on ranks was used. To isolate groups that differ from others, Dunn's method was used (both evaluations were done with Sigma Stat™ statistical software, version 1.02). In addition, the Mann-Whitney U test on Statgraphics software, version 5.2, was used to compare different groups of individuals. A P value of ≤ 0.1 was defined to indicate a tendency and $P \leq 0.05$ to indicate significance.

RESULTS

The comparability of the retention was tested with both the 1 µg/ml and the 3 µg/ml concentration on samples from a suitable subgroup of 26 patients (AML and ALL). The 1 µg incubation was associated with an ara-CTP retention of 41 ± 22%, and the 3 µg incubation with 48 ± 26%, the regression analysis yielded in r = 0.9. As a consequence, the results obtained with the two modifications of the assay were not differentiated in the following evaluations (Fig. 1, top).

The comparison of bone marrow and peripheral blast cells was performed in 15 children (9 ALL, 6 AML). The mean ara-CTP retention in bone marrow blast cells was

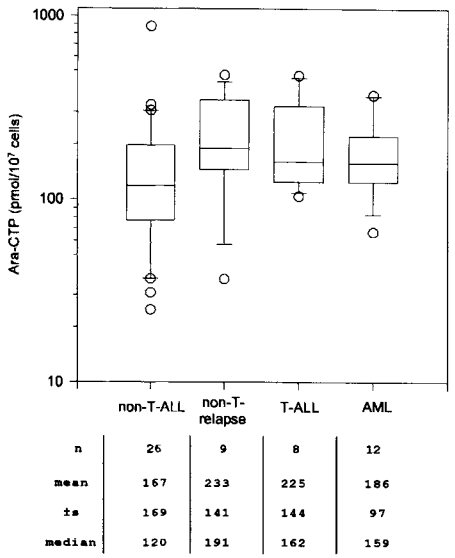

Fig. 2. Capacity of ara-CTP formation. Ara-CTP concentration (pmol/10^7 cells) following 1 hour incubation with 1 μg/ml ara-C.

45 ± 28% and in peripheral blast cells it was 48 ± 31%, and the correlation coefficient was r = 0.9. A distinction with regard to the source of the malignant cell clone was therefore deemed unnecessary (Fig. 1, bottom).

The maximum ara-CTP levels formed following 1 hour of incubation with 1 μg/ml ara-C (ara-CTP formation) were associated with a wide range of cellular ara-CTP concentrations, but significant differences between T-ALL, non–T-ALL, relapsed ALL, or AML were not observed (Fig. 2; $P = 0.23$). The ara-CTP formation following incubation with 3 μg/ml was significantly higher (not shown in Fig. 2). Linear regression analysis of the ara-CTP formation in 22 ALL samples (21 patients) incubated at 1 μg/ml (x) as well as at 3 μg/ml (y) yielded $y = 2.4 \times x - 39$ (r = 0.94). Due to a cell count too low for incubation at 1 μg/ml, nonmalignant bone marrow cells could not be included in the evaluation of ara-CTP formation.

Intracellular ara-CTP retention was comparable in common-ALL (67 ± 25%, median 66%, n = 25 at initial diagnosis) and the pre–B-ALL subtype (67 ± 26%, median 59%, n = 8 at initial diagnosis) in the subgroups of non–T-ALL. However, the results in non–T-ALL were markedly different from T-ALL, AML, and normal mononuclear bone marrow cells (Fig. 3, Table I). Statistical differences were found between AML and non–T-ALL, and between T-ALL and non–T-ALL (Kruskall-Wallis: $P < 0.05$, U- test: $P < 0.002$). The difference between newly diagnosed non–T-ALL and relapsed non–T-ALL showed a tendency towards lower ara-CTP-retention in the latter group (U-test, $P < 0.07$).

The ara-CTP retention was 34 ± 18% in 14 children with newly diagnosed AML and 26% and 18% in two

TABLE I. Ara-CTP Retention in Leukemic Blast Cells and Mononuclear Cells From Normal Bone Marrow

	x ± s	Median	Range	n
Normal BM	44 ± 24%	37%	22–55%	7
Non–T-ALL	67 ± 25%	64%	26–130%	33
Non–T relapse	51 ± 16%	52%	32–60%	11
T-ALL	37 ± 15%	31%	20–60%	8
T-ALL relapse			29%/15%	2
AML	34 ± 18%	29%	9–64%	14
AML relapse			26%/18%	2

BM = bone marrow.

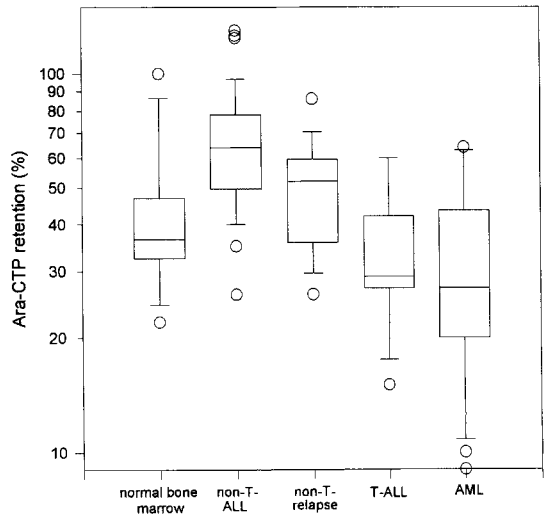

Fig. 3. Ara-CTP retention in normal bone marrow, initial non–T-ALL, relapsed non–T-ALL, T-ALL, and AML (relapses included, for detailed date, see Table 1).

children with relapsed AML. The distribution of these 16 observations was ≤15% in two, 15–29% in eight, 30–44% in two, 45–59% in two, and ≥60% in two. In eight children the ara-CTP concentration in the leukemic blast cells could be monitored in vivo during conventional continuous ara-C infusion (Fig. 4). The mean of all concentrations depicted in Figure 4 was 70 ± 69 pmol/10^7 cells.

No change in cellular ara-CTP retention was observed over the course of the continuous infusion: In four children the assay was repeated 36–44 hours after initiation of treatment. The RT%s (initial/36–44 hr) were 20%/30%, 29%/20%, 65%/65%, and 29%/35%. In two children, blast cells could be separated following the ara-C short-term infusion. Thirty minutes after the end of the infusion, the cellular ara-CTP levels were 181 and 92 pmol/10^7 cells. The corresponding trough levels dropped below the limit of detection.

DISCUSSION

Among all BFM treatment protocols, AML induction therapy is the only one that starts treatment with continu-

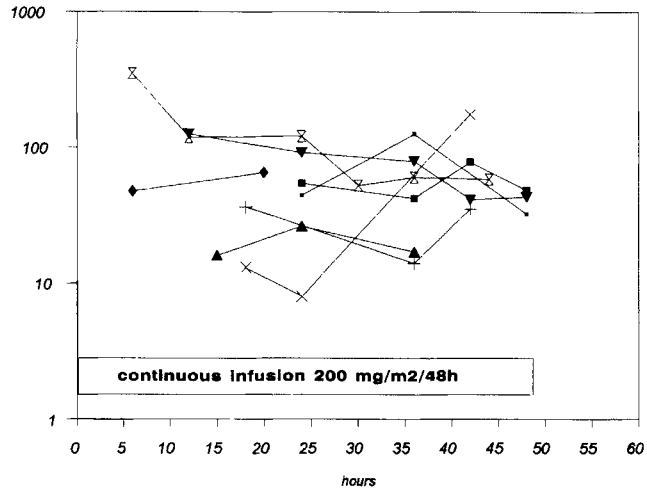

Fig. 4. Ara-CTP concentrations in myeloid blast cells during conventional continuous infusion therapy with ara-C.

ous ara-C infusion, a prerequisite for intracellular ara-CTP monitoring. The ara-CTP levels observed with this schedule varied widely (Fig. 4) and were slightly lower than those reported by Hiddemann et al. (median 26–96 ng/10^7 cells, q.e. 130 pmol/10^7 cells median) [23] or Rustum et al. (21–183 pmol/10^7 cells) [24]. The clinical relevance of interindividual variation of the cellular levels, however, is speculative. A relationship between clinical response and cellular ara-CTP levels under the conditions of conventional continuous infusions has not yet been systematically investigated. Target levels were always defined on the basis of high-dose ara-C treatment and, therefore, were disproportionately high: Plunkett et al. proposed a minimal effective ara-CTP trough level of 75 μM (≈200 pmol/10^7 cells) following high-dose (HD) ara-C short-term infusion [12,25]. Estey et al. observed a median steady-state level of 122 μM in responding patients and of 63 μM in nonresponding patients with HD continuous infusion [26]. These target steady-state levels were comparable with the peak values of short-term ara-C infusions with the conventional dosage. In two children we determined peak levels of 181 and 91 pmol/10^7 cells.

These in vivo values were of the same order of magnitude as the cellular concentrations following 1 hour of incubation in vitro (186 ± 96 pmol/10^7 cells). Moreover, the relatively rapid disappearance within 12 hours in vivo is consistent with a low ara-CTP retention of 29% (median) in AML and corresponds to published half-lives of 1.7–2.5 hours [23]. Our data thus support the assumption that the pharmacokinetic findings on blasts in vitro do reflect ara-CTP metabolism during conventional ara-C treatment.

One objection to the described in vitro model may be based on the relatively high ara-C concentrations used (1–3 μg/ml). With both concentrations, however, cellular uptake was shown to be limited by the activity of the transmembraneous carrier system. The formation of ara-CTP was completely suppressed by coincubation with dipyridamole, an inhibitor of the nucleotide carrier system [22] (see earlier). The experimental conditions thus ensured carrier-mediated uptake and reliably reflected conventional ara-C therapy. In addition, ara-CTP retention with 1 and 3 μg/ml ara-C was similar (Fig. 1). Single publications suggested a difference between cellular ara-CTP pharmacokinetics in bone marrow and peripheral blast cells [5,24]. Our findings do not indicate pharmacokinetic differences of in vitro ara-CTP retention between cells from these two sources (Fig. 1).

With a similar assay Rustum and coworkers reported a positive correlation between in vitro ara-CTP retention and remission duration following conventional ara-C therapy [13]. The adverse prognostic significance of low ara-CTP retention, however, seemed to be reduced or even abrogated by the use of continuous ara-C infusion and HD regimens [28]. In the in vivo investigation of Plunkett and others, the elimination rates and corresponding trough levels were significantly higher in responding patients [12,25], while the ara-CTP peak levels were comparable. In another investigation, the cellular ara-CTP pharmacokinetics (AUC, Cmax, t½) obtained with an initial high-dose ara-C infusion did not correlate with response duration in 147 AML patients on different ara-C therapy schedules. However, the steady-state concentrations on HD continuous infusion (6 g/m^2/96 hr) were significantly lower in refractory disease [26]. The degree of ara-CTP formation in vitro appeared to be even higher in relapsed AML than in previously untreated patients [28]. Overall, data relating cellular parameters of ara-CTP pharmacokinetics and clinical outcome have been conflicting. Peak levels seem to be of minor relevance compared with the duration of exposure to the triphosphate metabolite. During continuous infusion of ara-C, the ara-CTP steady-state levels in circulating blast cells increased proportionately to the ara-C application rate [14].

Publications on cellular ara-CTP kinetics in ALL have been rare. In adults, the ara-CTP elimination in lymphoblasts (n = 18) appeared slower than in myeloblasts (n = 51) [18]. Correspondingly, we found a higher ara-CTP retention in non–T-ALL (median 64%; see Fig. 2) than in AML (29%) for pediatric leukemias as well. In addition, our data clearly differentiate between non–T-ALL and T-ALL (31% RT).

According to the majority of studies, the T-cell phenotype has a poorer prognosis, compared with non–T-ALL, with cure rates much lower than 50% [29]. The list of biological features distinguishing between T- and B-lin-

eage leukemia cells is long. Several groups attempted to specify pharmacological properties of T-lymphoblastic cells as a basis for treatment alterations and studied ara-C metabolism in T cells. Momparler et al. [30] reported increased formation and a longer intracellular half-life of ara-CTP in different cell lines and an associated increased sensitivity to ara-C of T-lymphoid as compared with B-lymphoid and myeloid cells. Tanaka and coworkers investigated ara-C uptake and ara-CTP retention in a similar assay system using blast cells from adults in vitro [31] and described a 3.5 times greater intracellular accumulation and prolonged retention, which was 88% in T-ALL vs. 60% and 52% in AML and non–T-ALL. This finding correlated with a higher remission rate following treatment with mitoxantrone and continuous infusion of conventional ara-C.

Our own observations are inconsistent with these published findings. The cellular ara-CTP decrease was significantly more rapid in T-lymphoblastic than in non–T-lymphoblastic blast cells and was comparable with myeloid blasts (see Fig. 2). The kinetics in normal bone marrow samples was on the same order of magnitude as in T-ALL and AML blast cells. The ara-CTP formation did not differ between different leukemias, including relapse (see Fig. 2). These observations correspond to a report from Ross and coworkers, indicating that blast cells obtained from patients with nonlymphoblastic leukemia, responders as well as nonresponders under conventional ara-C treatment, formed comparable amounts of ara-CTP in vitro [32], and the ratio relating ara-C incorporation into DNA with cellular ara-CTP formation decreased with increasing ara-C concentration in the assay.

What are the implications for the discussion of the optimal ara-C schedule? Treatment with ara-C aims at ara-CTP formation, ara-CTP incorporation into the DNA, and DNA strand breaks. Ara-CTP incorporation and inhibition of DNA polymerase depend on relevant amounts of intracellular ara-CTP and the relationship between intracellular ara-CTP and dCTP [33]. An increase in cellular ara-CTP levels, however, will not automatically enhance cytotoxicity. The dose-response curve is apt to develop a plateau at a particular concentration, and any further increase will not necessarily result in increased cytotoxicity. In cell culture, cell kill correlates less significantly with cellular ara-CTP pools than with ara-C incorporation in DNA [34].

This observation might explain the fact that according to most publications ara-CTP peak levels failed to correlate with clinical data, while the ara-CTP elimination kinetics were, in fact, associated with clinical parameters [12,13,25,26,28]. Extending the time of exposure to a certain ara-CTP concentration, therefore, should be favored over an attempt to increase the maximum ara-CTP accumulation. Considering that even the ara-CTP formation with increasing ara-C dosages is a saturable process [5,27], the rationale of simply increasing the dose for ara-C treatment intensification is questionable. The prolonged ara-CTP elimination in non–T-ALL compared with mononuclear bone marrow cells (Fig. 3) may promise increased therapeutic gain and offers a rationale for short-term or push injection.

In T-ALL and AML there was no relevant difference between blast cells and bone marrow cells in our investigation. Effective treatment of the blast cell population, therefore, will be accompanied by corresponding bone marrow toxicity. This view is confirmed by the clinical observation that continuous infusion of conventional dose ara-C escalates bone marrow toxicity. The introduction of the ADE induction (48 hr continuous ara-C infusion followed by short-term ara-C, Daunorubicin, and etoposide) into the German AML-BFM studies resulted in a significantly increased event-free interval and reduced relapse rate [35,36]. In T-ALL the low ara-CTP retention offers arguments for the same strategy: Continuous infusion protects the cells from a decline in cellular ara-CTP, as we proved in patients receiving their first continuous infusion and then receiving the same dose as a short-term infusion (Fig. 4) according to the AML treatment strategy.

In comparison with our own results, Tanaka and Yoshida [31] found much higher ara-CTP retention in T-ALL. Still, their clinical observation of a 88% remission rate was based on continuous infusion of ara-C. In addition, Lauer and coworkers reported a selective effect of continuous infusions of ara-C during maintenance therapy: The combination cyclophosphamide/ara-C in addition to 6-mercaptopurine/methotrexate significantly improved relapse-free survival in children with T-ALL (7/18 = 36% vs. 0/8 = 0% after 30 months, $P = 0.015$) but not in non–T-ALL (35% vs. 48%) [37]. Over the last decade dose intensification has predominantly been realized by short-term infusion schedules with high doses of ara-C. High plasma levels of ara-C are expected to overcome cellular resistance by reduced expression of the nucleoside transport system via passive diffusion. As pointed earlier, high peak ara-CTP levels are not the main goal, but they do influence the AUC and the time above defined trough levels. Without a doubt, HD treatment resulted in enhanced toxicity and improved clinical outcome [1–3]. This might be due, in part, to the normally longer infusion duration of 2–4 hours in HD regimens and the much longer maintenance of therapeutic plasma levels compared with push injection of 30-minute infusion of conventional dosages. Our data provide the pharmacological basis for demonstrating that infusion duration is an essential variable in ara-C treatment schedules and that continuous infusion as an intensification strategy is a rational alternative or is even superior to HD treatment, especially in T-ALL and AML. Nevertheless, the dose of

continuous ara-C schedules is the second important variable that has to be optimized and can perhaps be custom tailored [14] in the future.

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